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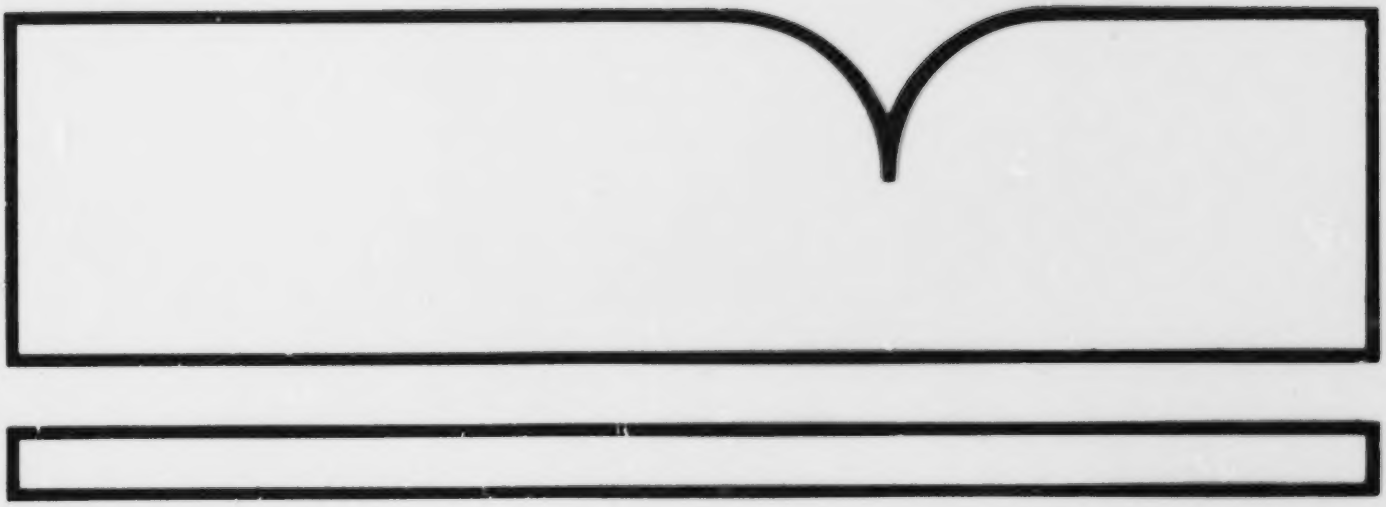

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by

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CHEMICAL EXPOSURES AND ANIMAL ACTIVITY: UTILITY OF THE FIGURE-EIGHT MAZE

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I. INTRODUCTION

In 1898, Colin Stuart described a series of experiments in which motor activity was used to evaluate the behavioral effects of dietary exposure to alcohol (1). To my knowledge, this is the first reported use of motor activity in behavioral toxicology. Since then, motor activity has been extensively used in both behavioral pharmacology and toxicology. The need for rapid and inexpensive tests in toxicology has prompted several advisory panels to recommend motor activity as a screen for neurotoxicity (2,3). Since motor activity reflects the general status of the organism, disruption produced by a variety of neurotoxic and non-neurotoxic mechanisms should be manifest as changes in motor activity. Studies of motor activity are useful, therefore, when no a priori reason exists for predicting a particular behavioral effect of exposure to a chemical. Activity measurements are also useful in characterizing the toxic properties of a chemical, such as onset and duration of action, reversibility of effect, etc.

The Environmmental Protection Agency (EPA) has recently developed test guidelines for the conduct of motor activity measurements (4). Since motor activity is a complex behavior and influenced by a variety of factors (5,6,7) these guidelines specify certain test conditions to insure the reliability of test data. In addition, they specify several criteria which govern the selection of a test method. Although numerous methods are available for measuring motor activity in laboratory animals (for reviews, 6,7,8,9,10), I will focus on one particular method, the figure-eight maze, and discuss several salient features of this test method in the context of EPA's Test Guidelines (4).

II. The Norton Figure-Eight Maze.

A. Configuration. In 1975, Norton, Culver and Mullenix described a figure-eight (residential) maze for monitoring motor activity in grouped rats (11, 12). The test chamber consists of a series of interconnected alleys (10cm x

10cm cross-section) which form a figure-eight around a raised central area (Figure 1a); in addition, two blind alleys extend out from the "waist" of the figure-eight. The maze shape, size of the alleys, and central raised area were fashioned to mimic burrows which wild rats (Rattus Norwegicus) dig in their natural habitat (13). Eight photocells, one in each alley, are connected to mechanical counters such that one activity count is recorded each time a photocell is interrupted. Subsequent versions of the figure-eight maze have utilized microprocessors to monitor photocell interruptions thus providing greater flexibility in data acquisition (e.g., 14). This feature meets one criterion of the EPA Test Guidelines (4) which requires that the apparatus be automated.

B. General Patterns of Activity. Three separate patterns of figure-eight maze activity will be discussed: (1) exploratory-obtained during a 1-hr test session; (2) circadian-obtained during a 24-hr test session; and (3) developmental-obtained at various ages during development.

1. Exploratory activity. Experimentally naive rats exhibit high levels of motor activity when placed in the figure-eight maze (Figure 1b). Although the initial 5-min activity levels are similar for females and males, females generally show higher overall activity in a 1-hr test session (Figure 1b insert). Extending the session further reveals clear sex differences in activity (e.g., 4,12). In contrast to many other activity devices, the rate of habituation in the maze (i.e., the decrease in activity over time) is relatively slow (6). When maze activity has been directly compared to other measurement devices (e.g., open field), this extended habituation has enhanced the sensitivity of the maze in detecting toxicity (47) and in addition, provides a measure which will distinguish between a toxicant-induced change in overall activity levels and a change in the rate of habituation (20). Finally, 1-hr maze activity meets the EPA criterion (4) requiring activity to reach asymptotic levels within the last 20% of the test session.

2. Circadian rhythms. The figure-eight maze can also be used to make quantitative comparisons of the effects toxic substances have on circadian rhythms of activity (12,14,16,17,18,19,20,21). This is an important feature since: (1) rhythms are known to influence the behavioral response to a chemical, and (2) toxicity may be reflected in an alteration in the circadian rhythm of activity (7). Schneider and Norton (18), for example, utilized the figure-eight maze to study circadian differences in the locomotor response to acute administration of d-amphetamine. In other cases, activity measured during the nocturnal period of the cycle can amplify the effects of brain

TABLE 1. CHEMICAL AND PHYSICAL AGENTS STUDIED USING FIGURE-EIGHT MAZES.

A. Pesticides	B. Metals	C. Drugs
propoxur (47,48)	lead (34),P	amphetamine (11,18,34)
carbaryl (47,48)	cadmium (21, 24),P	morphine (11)
deltamethrin (48)	triethyltin (19,23),P	chlorpromazine (15)
cismethrin (48)	trimethyltin (20,44),P	phenytoin (46),P
chlordimeform (48)	manganese (51),P	
chlordecone (49)		D. Misc.
mirex (49)		
endrin (50), P		carbon monoxide (17),P
		x-irradiation (52),P
		globus pallidus lesions (16)

P-denotes perinatal treatment

damage. For example, nocturnal activity of adult animals exposed to triethyltin (TET) on postnatal day 5 (22) or adult animals receiving lesions of the globus pallidus (16) was consistently increased after several days in the maze with little or no persistent difference in diurnal activity.

3. Developmental Pattern. Norton et al. (12) reported age-related changes in both the overall level and circadian distribution of figure-eight maze activity. Since their original report, maze activity has been used to evaluate developmental toxicity following a variety of toxicant exposures (Table 1). Culver and Norton (17), for example, evaluated the neurotoxicity of early postnatal exposure to high levels of carbon monoxide (CO). Juvenile hyperactivity was present in CO-exposed rats between 4 and 8 weeks of age but had disappeared by 3 months of age. Adult exposure to CO also produced hyperactivity (albeit reduced in amplitude) but this effect was irreversible, indicating that behavioral compensation occurred following developmental exposure to CO but not following adult exposure. In contrast, early postnatal exposure to TET produced hyperactivity which persisted through eight months of age (19), whereas adult exposure resulted in hypoactivity (23). A similar qualitative difference between neonates and adults was reported following cadmium exposure (21,24). Studies indicate therefore, that in control animals, motor activity levels change as a function of age and that chemically-induced changes in behavior may be reflected in age-dependent changes in motor activity. Furthermore, figure-eight maze activity appears to provide a sensitive measure

of developmental neurotoxicity which supports its continued use in behavioral teratology.

C. Interlaboratory Reliability. For a test method to have general utility in behavioral toxicology, it must engender and measure behavioral baselines which are consistent across laboratories. In a recent collaborative behavioral teratology study, performed under the auspices of the National Center for Toxicological Research (25), six different laboratories measured the developmental profile of figure-eight maze activity during 1-hr test sessions (Figure 2). These data demonstrate a remarkable consistency in both the overall levels and the developmental profile of maze activity across the six laboratories and illustrate how maze activity provides a stable behavioral baseline against which to evaluate behavioral toxicity.

D. Group Size Requirements. The EPA Test Guidelines (4) specify that a test method must be capable of detecting a 40% change in the activity of treated groups compared to the control group(s) with 90% power at the 95% confidence level. Sensitivity of a test depends on both the normal variability between subjects and the group size (26). Since variability is a function of the method, this requirement ensures that testing is consistently performed with enough animals to detect a moderate change in activity; the greater the variability of the test system, the greater the number of animals required to reliably detect an effect.

It has been our experience that figure-eight maze activity has a coefficient of variation (CV=standard deviation/mean x 100) ranging from 20-30% for individual animals tested for 1-hr. When animals are tested in groups of four, which is routine for Norton and coworkers, variability is reduced to 10-30%; 23-hr measures reduce the CV even further, to 5-10% (11).

To determine the number of animals required to detect a 40% change in maze activity, power calculations were performed (27) using CVs ranging from 20-30%. Two design situations were considered: (1) a 2-groups design (i.e., where a single control group is compared to a single treatment group) and (2) a 5-groups design (i.e., where non-treated control and vehicle-control groups are compared to 3 treatment groups). For a 2-groups design, 3, 4 and 5 rats per group are needed for CVs of 20, 25 and 30% respectively, whereas for a 5-groups design (which is typical in dose-effect determinations) group sizes of 5, 7 and 10 are required for these same levels of variability. Consequently, experiments using 10 animals per treatment group provide the sensitivity needed for most experimental conditions. As a general rule, power calculations should be performed to define the group size requirements for detecting a given level of

toxicity thus reducing the probability of false negative results (i.e., Type II errors).

E. Response to Reference Compounds. The EPA test guidelines (4) also require the determination of dose-effect functions for reference compounds which affect motor activity. These data are intended to demonstrate the general sensitivity of a given test system and to guard against the inadvertent use of an activity measure which lacks adequate sensitivity to detect a toxicant-induced behavioral change. Two drugs are recommended as reference compounds, d-amphetamine to demonstrate increases in activity and chlorpromazine to demonstrate decreases in activity.

Acute dose-effect functions for d-amphetamine and chlorpromazine were determined in adult rats tested for 3 hr in figure-eight mazes. d-Amphetamine (0, 0.6, 1.2 and 1.8 mg/kg) was administered to male and female rats once a week for 4 wk using a Latin Squares design such that each animal received all dosages of drug. For chlorpromazine, male rats were given one of 4 dosages (0, 1, 2 or 4 mg/kg) using an independent groups design. As shown in Figure 3, d-amphetamine produced non-monotonic increases in activity; the range of effective dosages is similar to that reported for activity in other measurement devices (e.g., 28,29,30) as well as the figure-eight maze (e.g., 11,18,34). Chlorpromazine, on the other hand, produced a monotonic decrease in maze activity over a dosage range which has been previously reported to decrease activity in other devices (e.g., 31,32,33).

It was of interest to determine if amphetamine affected maze activity in a rate-dependent fashion, similar to what has been reported for other behavioral tests (35). Amphetamine data were therefore analyzed to determine if there was a relationship between baseline levels of maze activity and activity following drug. As shown in Figure 4, the effect of all dosages of d-amphetamine was inversely related to baseline activity, i.e., the greater the baseline activity level (saline) the smaller the response to d-amphetamine (amphetamine/saline). These data are similar to findings reported in the mouse (36).

There is a growing emphasis in behavioral toxicology on using pharmacological agents to probe for neurochemical substrates of toxicity (37). The rate-dependent effect of d-amphetamine on maze activity has important implications for this practice. Take, for example, a situation where toxicant exposure results in persistent hyperactivity. If these animals also show a difference, when compared to control animals, in their response to a pharmacological challenge, this latter effect (altered drug response) may be the

direct result of the former effect (elevated baseline activity levels). In this case, failure to take rate-dependency into account may lead to erroneous conclusions regarding the possible biochemical substrates of toxicity.

F. Spatial Patterning. In their initial description of the figure-eight maze, Norton et al. (11) reported on the manner in which rats utilize the available space in the maze. They concluded that "....maintaining the rats in a spatially structured environment tends to structure the behavior in a way appropriate to the space available and this tends to stabilize the activity recorded in any portion of the environment." One way in which rats normally structure their behavior in the maze is to distribute their activity more or less evenly in all the corridors. Experimentally-induced brain damage, however, can alter this spatial distribution. Norton (16), for example, found that globus pallidus lesions produced substantially greater activity increases in the figure-eight maze corridors than in the blind alleys.

Ruppert and coworkers subsequently reported that the spatial distribution of activity, as well as overall activity levels, could be affected by toxicant administration (20,44,45). Spatial distribution of maze activity was divided into two categories: that occurring in the figure-eight portion of the maze (hatched bars) and that occurring in the blind alleys (open bars). As shown in Figure 5, activity changes produced by different treatments are associated with differences in spatial distribution. d-Amphetamine, chlorpromazine and neonatal trimethyltin (TMT) exposure produced uniform changes in activity in all corridors of the maze. Globus pallidus lesions, adult TMT exposure and neonatal TET exposure, on the other hand, produced increases in activity in the figure-eight corridors, and little or no change in activity in the blind alleys.

An interesting correlate to these data (44) is that effects on spatial patterning of activity parallel the effects on accuracy of performance in a radial arm maze which requires the use of spatial memory (38, 39). Both neonatal TET (40) and adult TMT (41) decrease accuracy in the radial arm maze whereas neontal TMT (42) and amphetamine (43) do not. The remaining treatments (GP lesions and chlorpromazine) have not been evaluated in the radial-arm maze. From these data, it appears that treatments which disrupt spatial patterning of figure-eight maze activity also disrupt an animal's ability to use spatial cues to perform a spatial memory task. Further studies are needed to determine the extent to which spatial patterning in the figure-eight maze accurately predicts performance on a spatial learning task.

III. Summary

In 1975, Norton and coworkers (11) described a figure-eight maze system for measuring motor activity in rodents. Since their original report, a number of studies have appeared in the literature which have utilized the figure-eight maze to evaluate the behavioral toxicity of a variety of chemical and physical agents (Table 1). The present paper describes a number of salient features of the maze which have emerged from these studies which support its continued use in toxicological studies. These features are described in the context of EPA's Test Guidelines for motor activity (4).

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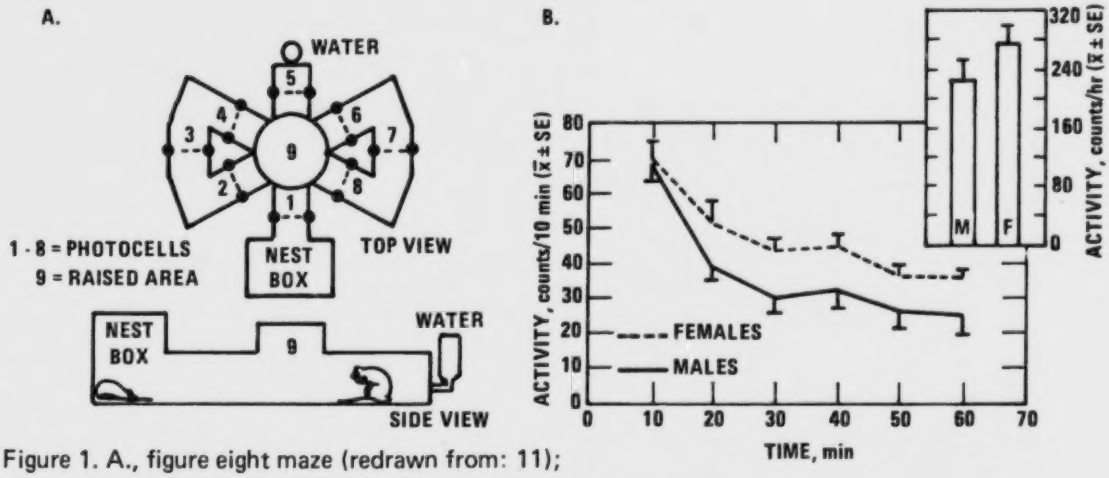

Figure 1. A., figure eight maze (redrawn from: 11);

B., one-hour maze activity for male and female rats (15).

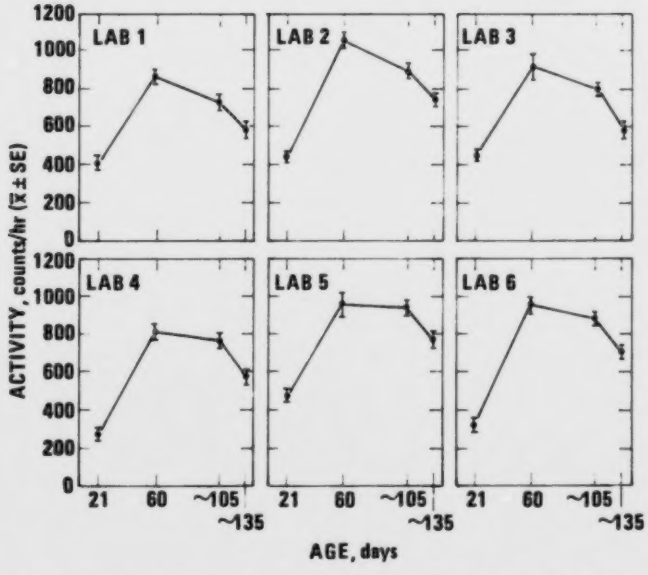

Figure 2. Developmental profile for one-hour maze activity in male rats (n=32/point). Unpublished data from NCTR/NTP Collaborative Study, published by permission. C. A. Kimmel, J. Buelke-Sam, and J. Adams.

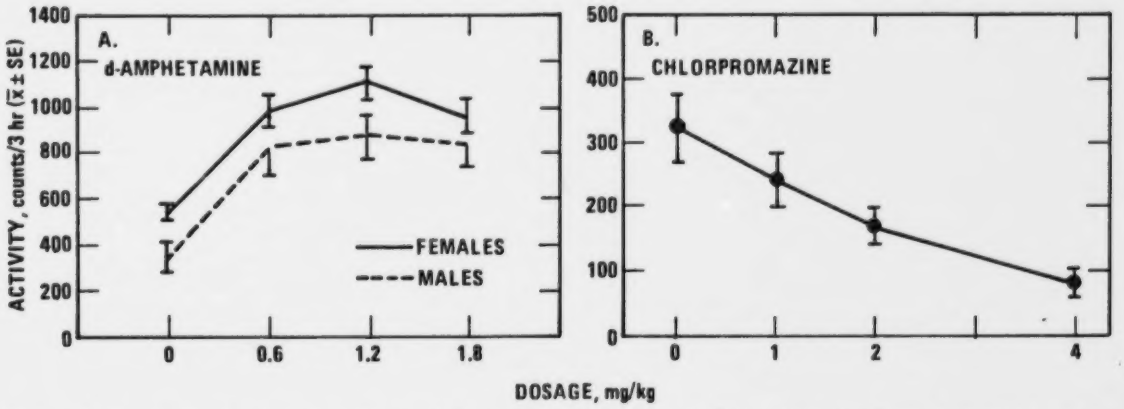

Figure 3. Dose-effect curves for figure-eight maze activity in rats following: A., d-amphetamine, and B., chlorpromazine (15).

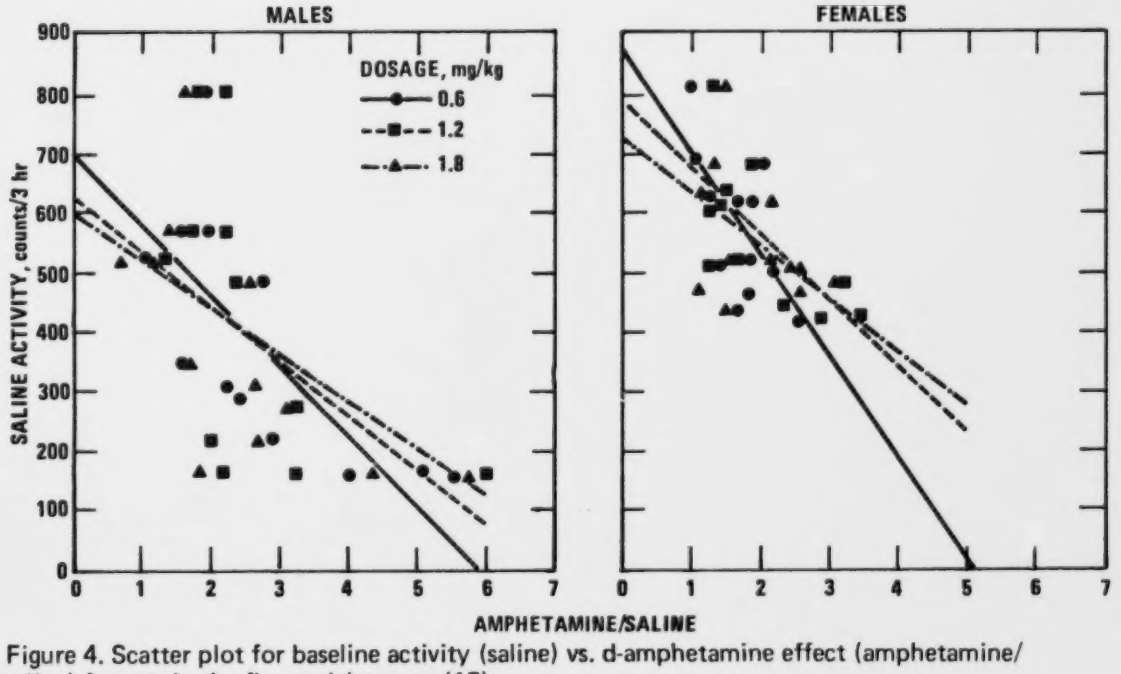

Figure 4. Scatter plot for baseline activity (saline) vs. d-amphetamine effect (amphetamine/saline) for rats in the figure-eight maze (15).

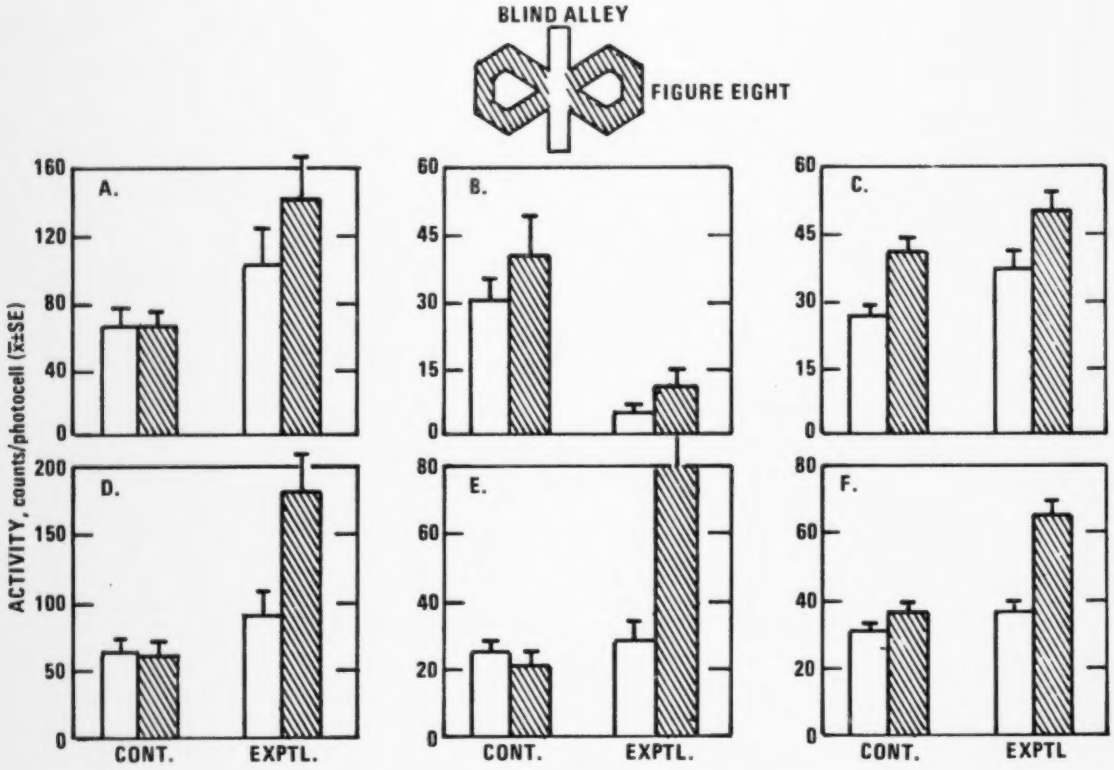

Figure 5. Spatial distribution of figure-eight maze activity in adult rats following six different treatments: A. d-amphetamine, 1.2 mg/kg, 3-hrs, females (ref. 15); B. chlorpromazine, 4 mg/kg, 3-hrs, males (ref. 15); C. neonatal trimethyltin, 6 mg/kg, 1-hr, males and females (ref. 44); D. G-P lesions, nocturnal activity, females (ref. 16); E. trimethyltin, 7 mg/kg, 1-hr, males (ref. 20); and F. neonatal triethyltin, 6 mg/kg, 1-hr, males and females (ref. 45).